

Lemons, rods and turreted balls: INL sequences Yellowstone viruses

By Rachel Courtland, INL science writer

Four years ago, a blast of superheated subterranean water burst through a tree-covered hillside in Yellowstone National Park, creating a patch of bubbling ponds, boiling springs and steaming fumaroles. Like many of the thousands of inhospitable thermal features scattered about the park's caldera, it didn't take long for an entire ecosystem of microbes to take root. Along with them came a number of unusual viruses. The question, for INL microbiologist Frank Roberto, is how.

In many Yellowstone hot springs, a sulfur-eating microbe called *Sulfolobus* thrives in the acidic, mineral-rich water. But *Sulfolobus* is not without predators. For a host of viruses, incapable of surviving by themselves in the pools, these microbes are idyllic islands in an acid ocean. It's an unexpected relationship for such an extreme environment, leaving researchers wondering how these viruses came to be, how they reproduce and why the same kinds seem to pop up in geologically-isolated places.

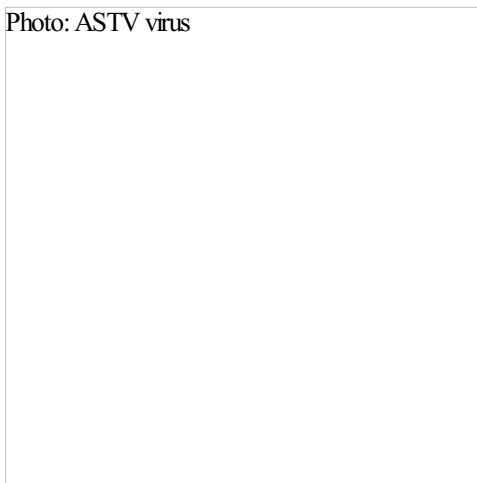
"It's really a mystery how these viruses could have evolved if they can't survive in hot pools by themselves," says Roberto, who works to sequence and analyze virus DNA. "To reproduce, these viruses need to leave their hosts. Then they're entering a really hostile environment."

Now, years of sampling and study are beginning to pay off. Researchers at the Montana State University Thermal Biology Institute, with the help of Idaho National Laboratory, are learning more about these unique viruses, their capabilities, and their role in the Yellowstone hot springs ecosystems.

Deep Mysteries

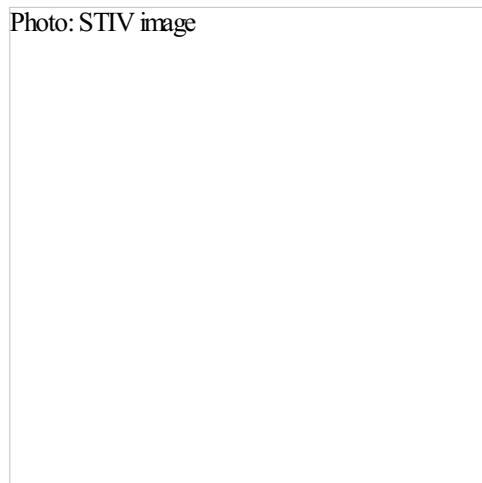
On the walk to the new thermal features by fishless Nymph Lake, the late-summer sun competes with smelly clouds of sulfurous steam. Roberto and MSU researcher Alice Ortmann set down their gear by a pool of clear water, bubbling with dissolved gas emerging from deep underground. Sitting down near the edge, Ortmann drops a pH and temperature probe down into the pool. The water's temperature is just under boiling, with the same acidity as stomach acid. Such conditions are common for Yellowstone hot pools. A big question for biologists is how much microbes and their predators contribute to creating this environment. "We know microbes can have a big impact on geochemistry," Roberto says, "affecting everything from the amount of planetary carbon to the acidity of water leached in rock mining." Viruses that infect hot pool microbes may have a similar effect on their environment by keeping certain populations in check.

Photo: ASTV virus



A) Tunneling electron microscope image of ASTV viruses isolated in Yellowstone.

Photo: STIV image



B) Computer-reconstructed image of STIV, from the Yellowstone's Rabbit Creek area. Images courtesy of the Thermal Biology Institute at Montana State University.

Set to grow in the laboratory, microbes like *Sulfolobus* will adjust the acidity of the surrounding water to their comfort level. But in Yellowstone, studying the effect of even a single thermal pool ecosystem on its environment is tricky. Pools are rife with microbes and viruses with unknown capabilities.

What's more, researchers can't see underneath pools, so it is difficult to know how deep life persists and what forms it takes. Dig down 300 feet near Old Faithful, and the temperature can top 650 degrees Fahrenheit, hot enough to melt elements like lead and sulfur. But each pool's water has a different pathway to the surface. Mud pots of different colors, chemistries and temperatures can be inches apart. "It's hard to measure how far down microbes go or what the ecosystem's like there," Ortmann says. "We're only really scratching the surface."

Steam Locomotion

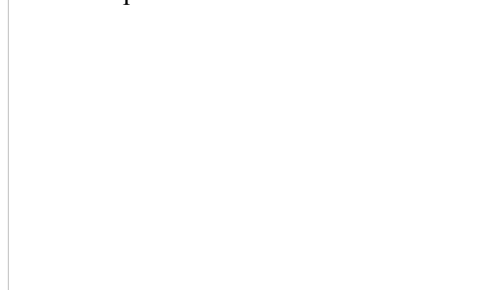
Researchers suspect viruses may play a key role in changing the fundamental chemistry of the hot pools by controlling microbe populations. To investigate the impact of viruses on their ecosystems, in 2002, MSU researchers began visiting Yellowstone every month for two years to sample three distant hot pools. The virus DNA in the samples was then sequenced at INL. Initially, Roberto says, the team looked for a relationship between pool conditions and microbe population. Over the course of the study, some microbe species populations fluctuated wildly. The biologists expected pool conditions might change to match, but the acidity of the pools stayed the same.

Instead, the researchers found something surprising - changes in *Sulfolobus* virus populations suggested they were migrating from pool to pool. Since subterranean water temperatures were so high, underground migration seemed unlikely. Following a hunch, the researchers isolated viruses in the air columns above pools and concluded the viruses must be buoyed from pool to pool in droplets of steam. The results of this research are scheduled to be published in an upcoming issue of the Proceedings of the National Academy of Sciences.

Strangely, viruses thrived in pools even if their chosen hosts were relatively rare. *Sulfolobus* viruses are not hearty. Most survive only a few hours in the acidic water outside a host. If *Sulfolobus* is not one of the more common types of microbes in a hot pool, a virus' next victim might be too far away to reach. Still, the study suggests the viruses are successful in populating microbes, even if new hosts are rare and separated by hostile waters.

A larger understanding of the significance of viruses in Yellowstone thermal features is still on the horizon, Roberto says. "We're in uncharted territory in terms of understanding how these viruses impact the ecosystem of these pools," he says. When viruses leave their hosts, they sometimes carry bits of host DNA with them. Roberto speculates Yellowstone viruses may transport genetic information from one pool to another, impacting the evolution of microbes across the park.

Photo: Red pool



Iron-reducing microbes may contribute to the red color of this pool near Norris Geyser Basin.

Unknown capabilities

With thousands of thermal features to sample, hot pool surveys have yielded some unexpected findings about the diversity of life in Yellowstone. *Sulfolobus* viruses vary in shape, although most resemble rods, balls and lemons. But in 2004, researchers found an entirely novel-looking virus in the Rabbit Creek area of the park. *Sulfolobus* turreted icosahedral virus (STIV) is spherical, with 12 bumpy square turrets protruding from its outer protein shell. No one has yet determined what the virus' turrets are designed for, although microbiologists suspect it may be used to attach to host cells or transfer genetic material. In addition to analyzing its unique shape, Roberto and his colleagues are still struggling to analyze STIV's genome. The function of 40 percent of STIV's DNA is unknown.

Even more surprising, proteins in the STIV's coat bear an unexpected resemblance to bacterial and animal viruses. This suggests STIV carries some ancient characteristics of a common

ancestor, preceding the first major divergence of life more than three billion years ago. "STIV is interesting because it doesn't resemble other viruses," Roberto says. "It seems to be at the crossroads, with features that are common to viruses in all domains of life." Mysteriously, despite recent discoveries about virus migration, STIV has only ever been found in one pool in mud pot-dominated Rabbit Creek. "That's been one of the interesting things," he says. "It's been very difficult to find again."

Beyond the Park

Studying geochemistry is only one goal of virus research, Roberto says. As thermophilic microbes are being adapted for a number of energy uses, from cleaning the smokestacks of coal plants to processing cellulose for ethanol, understanding how viruses interact with their hosts may become increasingly important. Since viruses can stay dormant in microbe populations and have a proven ability to migrate; large-scale industrial applications that use microorganisms will have to take potential infections into account.

But viruses also have some advantages. "Viruses have provided a stable way to introduce new genetic traits into susceptible hosts," Roberto says. Introducing a specially-selected virus could be a way to tailor thermophilic hosts for a wide variety of applications.

To get there, Roberto and his MSU collaborators need to get a better idea of what entire microbial-viral ecosystems look like. So the team is in the process of collecting hot pool samples to send to the DOE Joint Genome Institute, which will sequence all the DNA. Then the hard work begins: piecing the wealth of code back to find the genomes of all the organisms in each pool. From there, Roberto hopes, the team will have snapshots of all the microbes and viruses in individual pools, a window into their capabilities, and a better chance at understanding how they interact.

Sampling the sites

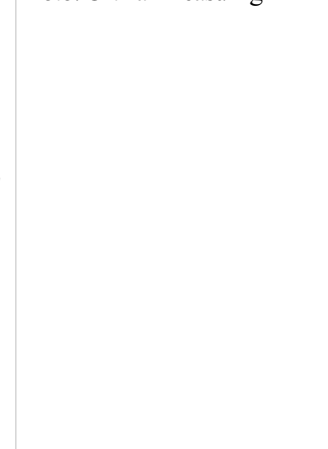
Yellowstone field work is not without its hazards. While many of the thermal features researchers sample are just off the road, a number involve lengthy hikes and unstable footing. Depending on the season, researchers may encounter grizzly bears. Snow can easily hide pools from view. On one winter expedition, Roberto recounts, he came across a herd of wild bison huddled around hot springs for warmth. "It's the kind of situation where you're just hoping they're in a good mood that day," he says. Still, Roberto says the risks are outweighed by the rewards. "I guess researchers' dirty secret is that we love this field work," he says, days before heading out for another visit to the park. "There's no place we'd rather be."

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Photo: Ortman measuring



MSU microbiologist Alice Ortman takes measurements on a mud pot near Norris Geyser Basin.